Metabolomics Strategies Using GC-MS/MS Technology

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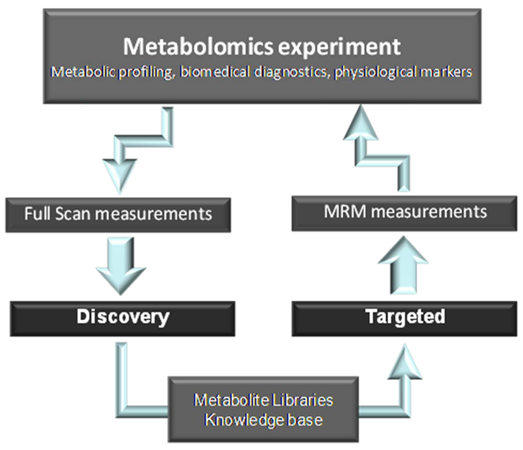
Introduction

The physico-chemical diversity of biological small molecules makes a metabolomic analysis very difficult. Therefore, different analytical strategies are necessary and are ideally combined with each other. Analyses focusing on a group of metabolites or watching as many metabolites as possible at specified environmental or developmental stages is called metabolite profiling. On the other hand metabolite target analyses are restricted to specific metabolites of interest, which can be selectively monitored and quantified. There is a discrepancy of detectable peaks in a typical metabolomics sample and the number of assignable chemical identifications due to a restricted availability of non-complete reference libraries and technical problems such as ultracomplex coelution of compounds and non-optimal mass spectral deconvolution in gas chromatography-mass spectrometry. Therefore, we propose an integrative approach combining advantages of targeted analysis using multiple reaction monitoring (MRM) and full scan measurements to enhance the selectivity of the analysis, increase the number of identifiable and selectively quantifiable chemical structures and allow for absolute quantification strategies.

We investigated one of the key model systems for energy genomics *Populus* sp. and its interaction with growth promoting endophytes. Although we have the full genome sequence, we cannot predict dynamic metabolic phenotypes. Here, metabolomics represent a key-technology to reveal the genotype – phenotype interaction [1].

FIGURE 1. The 2-tier strategy of combining full scan MS-analyses of metabolites and targeted analysis. Full scan MS-analysis provides a completely unbiased identification of metabolites and metabolite dynamics. MRM measurements provide the targeted quantitation using the identical instrument platform.



In this poster the central strategy in metabolomics is described using GC-MS/MS technology in a 2-tier overall analysis to develop a highly sensitive and selective method for the detection as well as absolute quantification of metabolites using the triple stage quadrupole GC-MS/MS-MRM-technology.

First, a semi-quantitative discovery phase identifies as many metabolites as possible from biological samples in a complete unbiased manner using full scan mass spectra acquisition. Second, the discovery phase is followed by a target phase, where metabolites of interest can be selectively measured and quantified absolutely.

Methods

Sample Preparation

Extraction and derivatization of plant metabolites was done slightly modified according to [2].

GC-MS/MS Conditions

A mass spectrometer Thermo Scientific TSQ Quantum GC triple quadrupole system was used, equipped with a Thermo Scientific TRACE GC Ultra and Thermo Scientific TriPlus Autosampler.

Split/splitles inj.	230 °C, splitless mode, splitless time 2 min, split flow 10 mL/min
Injection:	1 µL
Oven program:	Initial: 70 °C hold 1 min Ramp 1: 1 °C/min to 76 °C Ramp 2: 6 °C/min to 330 °C, hold 5 min Post-run: 10 min at 325 °C
Transferline	340 °C
Column:	TR 5MS SQC, 5% Phenyl (equiv.) Polysilphenylene-siloxane 15 m × 0.25 mm ID × 0.25 µm
Carrier gas:	He, constant flow at 1 mL/min
Ionization:	EI, positive ion
Emission :	100 µA
Ion Source:	250 °C
Scan Mode 1:	Full Scan (m/z 40-600), scan time 250 ms
Scan Mode 2:	MRM (Multiple Reaction Monitoring)
Mass resolution:	Q1 0.7 Da (FWHM)
Collision:	CID (Collision Induced Dissociation)
Collision Gas:	Argon, 1 mTorr

TABLE 1: Selection of MRM-transitions for quantitation of metabolites. Using more than one transition confirms the identity of a peak and gives the possibility to select a matrix-dependent quantifier-transition. For a higher amount of SRMs it is crucial to use scheduled MRM methods in order to ensure sufficient data points for peak integration.

Metabolites	Derivatives	Precursor m/z (Q1)	Product 1 m/z (Q3)	Product 2 m/z (Q3)	Product 3 m/z (Q3)	Collision Energy [V]
Alanine, L-	2 TMS	116.02	73.03			10
	2 TMS	117.01	73.80			10
	2 TMS	190.07	147.00	130.90		10
	3 TMS	99.99	84.65	59.00		10
	3 TMS	188.10	99.80	113.80	59.00	10
	3 TMS	262.15	132.60	113.50	99.70	10
Arbutin	5 TMS	254.15	239.00			20
	5 TMS	361.20	243.00			15
	5 TMS	450.20	216.90			15
Asparagine, L-	3 TMS	115.92	73.00			10
	3 TMS	131.93	114.60			10
	3 TMS	187.99	73.00	130.90		10
	3 TMS	231.06	115.78	131.80		10
Aspartic acid, L-	3 TMS	188.11	99.75	132.75		10
	3 TMS	202.05	132.60	127.80		10
	3 TMS	232.12	99.80	116.73	187.90	10
Fructose, D(-)	5 TMS, 1MeOx	307.12	103.02			18
Glucose, D(+)	5 TMS, 1MeOx	319.15	129.00			18
Glutamic acid	2 TMS	174.09	84.00			10
	3 TMS	246.13	127.90	155.90		15
Glutamine	3 TMS	245.20	156.00			15
Indole-3-acetic acid	2 TMS	319.21	202.24			10
Isoleucine	2 TMS	158.10	73.00			15
Leucine, L-	2 TMS	158.13	102.00			15
Tryptophan, L-	2 TMS	131.90	116.80			10
	2 TMS	202.10	73.00			10
	2 TMS	231.05	117.60	99.80		10
	3 TMS	202.10	73.00			10
	3 TMS	291.10	100.70	128.80	85.70	10
Lysine	3 TMS	200.12	82.00			18
Malic acid	3 TMS	233.14	73.03			18
	3 TMS	307.13	147.00			20
Mannitol	6 TMS	345.19	255.12			19
Myo-Inositol	6 TMS	190.98	73.00	102.85		10
	6 TMS	265.00	220.80			10
	6 TMS	305.03	216.90	142.80	132.80	10
	6 TMS	318.02	214.85	302.89	220.84	10
Phenylalanine, L-	2 TMS	192.10	73.00	99.70		10
	2 TMS	218.09	129.80	99.77		10
Ribitol	5 TMS	205.20	147.03	117.03		14
	5 TMS	217.00	129.01			15
	5 TMS	307.00	103.04	217.15	129.00	18
	5 TMS	319.17	129.05			18
Salicin	5 TMS	243.10	155.00			15
	5 TMS	271.10	154.80			15
	5 TMS	361.10	243.05	168.77		20
Salicylic acid	2 TMS	266.90	209.00	73.08		10
	2 TMS	267.00	249.00	193.00		10
Shikimic acid	4 TMS	188.96	73.00	132.70		10
	4 TMS	204.00	188.80	132.75	162.80	10
	4 TMS	255.02	238.82	132.66	222.82	10
Sucrose, D(+)	8 TMS	271.90	73.04	155.00		20
	8 TMS	437.14	256.99	190.92		20
Trehalose, alpha,alpha'-	8 TMS	217.10	129.00	73.00		15
	8 TMS	361.10	169.00	243.00	270.80	15
Valine	2 TMS	144.10	73.00			10

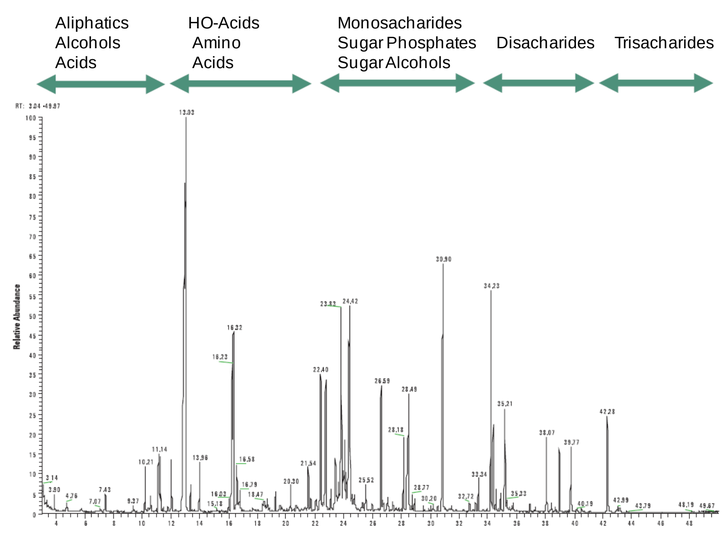
Workflow Phase I: Discovery

The discovery phase provides the identification of as many metabolites as possible by GC/MS Full Scan analysis. This phase is dominated by deconvolution of the chromatograms to extract the full and representative mass spectra for subsequent compound identification (Figure 2).

The deconvolution step is greatly facilitated by using the AMDIS program and the NIST mass spectral library search program. Unique compound mass spectra are extracted by the analysis of all the transient ion signals allocating ion masses and relative intensities for each eluting compound.

Identification of metabolites is based on characteristic EI fragmentation patterns as well as on retention time (RT). The mass spectrum identification is facilitated by searching large data bases with NIST, Wiley or dedicated collections of mass spectra like GMD, Fiehn-Library and in house metabolite databases (MOSYS database of TMS-derivatized metabolites and MRM transitions). LCQuan (Thermo Scientific) is used for relative quantification by peak integration of specified quantification ions. In Figure 3 an analysis of poplar leafs is shown either grown with growth- promoting endophytes or no presence of endophytes [3].

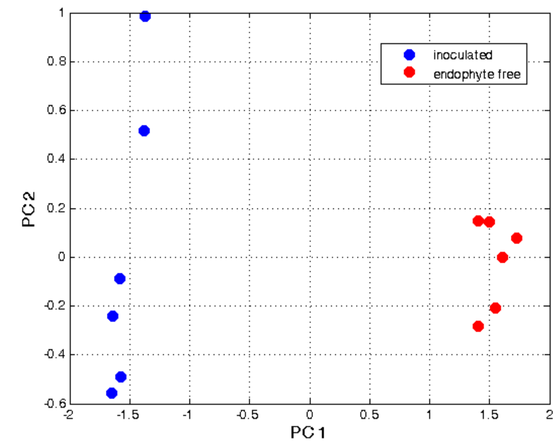
FIGURE 2. A complex metabolite profile measured in the full scan mode of the TSQ Quantum GC instrument. Chemical classes are assigned to the corresponding chromatographic regions.



References

1. Weckwerth, W., *Unpredictability of metabolism - the key role of metabolomics science in combination with next-generation genome sequencing.* Analytical and bioanalytical chemistry, 2011. **400**(7): p. 1967-1978.
2. Weckwerth, W., K. Wenzel, and O. Fiehn, *Process for the integrated extraction identification, and quantification of metabolites, proteins and RNA to reveal their co-regulation in biochemical networks.* Proteomics, 2004. **4**(1): p. 78-83.
3. Scherling, C., Ulrich, K., Ewald, D., and Weckwerth, W. (2009). A metabolic signature of the beneficial interaction of the endophyte paenibacillus sp. isolate and in vitro-grown poplar plants revealed by metabolomics. Mol Plant Microbe Interact 22, 1032-1037.

FIGURE 3: Sample pattern recognition using principal component analyses (PCA). Endophyte-free poplar plants can be clearly distinguished from inoculated ones.



Workflow Phase II: Targeted Quantitation

For the targeted workflow phase II suitable MS/MS transitions for each compound are used. The selected precursor ions get fragmented to structure specific product ions in the collision cell of the triple quadrupole MS. The integration of the product ion peaks provides the selective quantitation of all target metabolites.

Figure 4 shows the chromatograms of IAA and glucose compounds at the level of 50 pmol injected amount. Both compounds although coeluting can be integrated independently from each other due to the different SRM transitions used.

FIGURE 4. Coeluting IAA and glucose with separate product ion mass traces for individual quantitation.

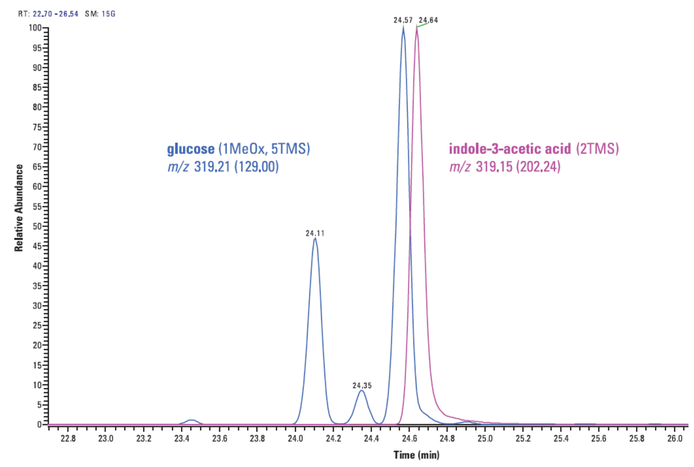
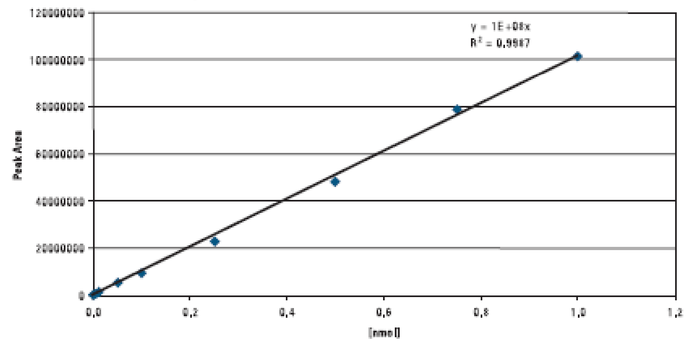


FIGURE 5. Linear quantitation calibration for Glucose



For glucose a wide linear calibration range could be achieved from 1 fmol to 1 nmol on column, stretching over 18 levels with 6 orders of magnitude with excellent precision of $R^2 = 0.9987$, see Fig.5.

Conclusions

GC-MS/MS with the TSQ Quantum provides both metabolomic workflow phases on one instrument platform. This allows the application of both methods directly to the same sample providing highest convenience of sample handling and highest confidence with respect to technical variability:

Phase I: Discovery phase analysis

- Selective identification
- Fast full scan analysis with deconvolution
- Access to the largest mass spectral know-how bases
- Reference library building

Phase II: Quantitation

- Very accurate quantification
- High selectivity, high sensitivity by MS/MS mode
- High dynamic range for interesting metabolite markers
- Complex mixtures analysis using the MRM mode
- Coeluting compounds get separated and individually quantified by compound-specific MRM

The TSQ Quantum XLS is the optimal instrument for use in metabolomics profiling to perform the metabolite screening as well as for the targeted metabolite quantitation in complex samples for the analysis of hundreds of target metabolites in one GC-MS/MS-MRM run, and high sample throughput work .

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